Research Article

Techno-economic analysis of bioethanol production from microwave pretreated kitchen waste

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Abstract

Cost-effective production of bioethanol from waste material is becoming the need of the hour to combat the exhaustive nature of fossil fuel. In this study, bioethanol was produced from microwave-pretreated kitchen waste at high dry material consistency. Pretreatment was performed for 30 min at a constant power of 90 W. Liquefaction/saccharification was done with *in-house* produced amylase from *Bacillus licheniformis* MTCC 1483. The liquefaction step was optimized using response surface modeling. Three factors, viz. pH, concentration of dry substrate and amylase, were optimized by using reducing sugar and ethanol yield as response. The optimum conditions of input parameters obtained were pH 7.5, dry material 40% (w/v) and amylase 15 IU g^{-1}. The process developed in the present study leads to 0.129 g ml^{-1}, i.e., 0.32 g per g biomass ethanol production. The novelty of the manuscript lies in the fact that no acid/alkali hydrolysis was carried out for the release of reducing sugar. Instead, microwave treatment was carried out at low power for longer time so as to release maximum sugar. The cost incurred in bioethanol production was also estimated by taking cost of chemicals, instruments and operating cost in account. The total cost of bioethanol produced in the present study was calculated as 0.143 $/l of ethanol. A 8.32-fold decrease in price of ethanol produced in the present study was observed when compared to the market selling price of ethanol. This makes the developed process economically and industrially feasible.

Keywords Bioethanol · Kitchen waste · Microwave pretreatment · Liquefaction · Amylase · Cost analysis

1 Introduction

One of the major concerns in the world these days is the depletion of fossil fuels and the deterioration of the environment. Developed and developing countries are exploiting fossil fuels, such as oil, coal and natural gas, at increasing rate leading to their overall depletion. Hence, there is a desire to explore the likelihood of other energy sources that are as economical as oil and might be directly used as fuel or by mixing with present fuels [1]. Biofuels, the liquid or gaseous fuel produced from biomass, provide an ecofriendly alternative to meet growing energy needs. Various biofuels include biomass and biogas energy, primary alcohols like methanol and ethanol, vegetable oils, bio-diesel, etc. [2, 3]. These renewable fuels are expected to offer many benefits including sustainability, low greenhouse gas emissions, regional development, social construction and agricultural development [4].

Bioethanol, the product of fermentation of carbohydrates, can be used as biofuel as it has high octane number than gasoline and tolerates higher compression ratio [5]. It can be generated from any material containing starch or sugar. Previously, research was focused on first- and second-generation biofuels which utilizes sugar or starchy syrups and waste by-products, respectively [6]. But due to the economic constraint of using these resources, research is now going on for utilizing lignocellulosic waste as organic matter for bioethanol generation [7]. Tons of

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kitchen waste is produced in urban areas daily comprising of household waste, restaurants and hotels. In routine, kitchen waste is discarded as such in environment. Its disposal by burning creates lots of pollution due to emission of green house gases [7, 8]. Kitchen waste includes potato peels, onion peels, ginger peels, garlic peels and other seasonal vegetables and fruits which are routinely used in-house for food preparation. Kitchen waste can also be utilized for bioethanol production as it is having high sugar and starch content [9]. Therefore, in the current study, kitchen waste was chosen as raw material for bioethanol production.

The conversion of cellulose and hemicellulose into glucose always remains a challenge to the process, leading to increasing cost of the ethanol production. The acid/alkali process routinely acquired for the treatment of lignocellulosic waste leads to the degradation of soluble sugar under harsh conditions [10–13]. Microwave pretreatment is one of the hopeful choices because of its high effectiveness and ease of implementation [14, 15]. It has been shown in the literature that microwave irradiation can alter the structure of lignocellulosic waste by cleaving the bond between lignin, cellulose and hemicelluloses leading to increased enzymatic vulnerability, resulting in increased sugar recovery from enzymatic hydrolysis [16–18].

Therefore, in this study, kitchen waste was treated only with microwave radiation followed by enzymatic treatment with amylase. The peculiarity of the present work is that no acid/alkali treatment was done for the hydrolysis of waste. The treatment condition was standardized by using statistical methods, viz. response surface methodology (RSM). Percentage reducing sugar and ethanol produced were taken as responses. In the end, cost of production of bioethanol was calculated to study process economics.

2 Materials and methods

2.1 Raw material

The household waste used in this work was procured from the local canteen of Chandigarh Group of Colleges, Landran, Mohali, India. Kitchen waste contains peels of potato (50%), onion (20%) and seasonal vegetables (30%). Waste was collected during winter season for successive 30 days and dried overnight at 55 °C. Dried waste was ground in a mixer grinder to powder form and sieved through 5 mm mesh size sieve to obtain particles of uniform size. This was used as raw material throughout the study.

2.2 Chemicals and reagents

Di-nitro salicylic acid (DNSA) used in the present study was purchased from Sigma-Aldrich, St. Louis, USA. All other chemicals and reagents were obtained from HiMedia, India.

2.3 Characterization of kitchen waste

Kitchen waste was characterized in terms of its pH, moisture content, total solids, volatile solids, ash content, Kjeldahl nitrogen and total organic carbon as per method given by United States Environmental Protection Agency (USEPA) [19]. pH was measured using a standard calibrated pH meter (FE20, METTLER TOLEDO). 1 g of waste was dried in an oven at 105 °C in a crucible for 1 h. Moisture content was calculated by calculating the difference between initial and final weight. The amount of solids left after the moisture gets evaporated was recorded as total solids. The sample which was dried for total solids estimation was placed in muffle furnace at 585 °C for 4 h to estimate the amount of volatile solids. 1 g of sample was heated at 585 °C for 4 h to determine the ash content. Total organic and inorganic nitrogen was estimated by Kjeldahl standard method. Total organic carbon was calculated by Walkley–Black method of oxidation with chromate [20]. Total carbohydrates were analyzed by standard anthrone method by making standard curve of glucose [21]. Analysis of total protein was carried out by Lowry's method [22].

2.4 Bacterial culture and amylase production

The amylase was extracted from the culture of *Bacillus licheniformis* MTCC 1483 [23]. Sub-culturing of bacterial strain was done routinely to maintain the strain. Starch hydrolysis test was carried out to study the α-amylase production by bacterial strain. The plate was stained with Gram's iodine solution to visualize the zone of hydrolysis. Amylase was produced by solid-state fermentation with paddy straw as substrate [24]. Paddy straw was washed under running tap water for 2–3 times. After washing, paddy was treated with 1% NaOH to remove extra chemicals, soil or dust particle and lignin, etc., present in the straws. Paddy straw was ground and sieved through uniform mesh size sieve to obtain equal-sized particles. Fermentation was carried out under solid-state conditions at 37 °C with 40 g paddy straw. 160 ml of distilled water, containing 0.002 g NaCl, 1 g yeast extract and 100 µg Tween 80, was used for moistening the substrate. 10% (per gram of substrate) bacterial inoculum was used. After 48 h, the fermented media was mixed with 50 ml phosphate buffer pH 7.4 to extract the crude enzyme. The slurry was squeezed through a muslin cloth. Centrifugation was done at 10,000 rpm for 15 min to obtain culture supernatant.

2.5 Enzyme assay

Estimation of amylase was carried out by dinitrosalicylic acid method at 50 °C pH 8.0 [25]. One unit of amylase activity was defined as the amount of the enzyme that catalyzes the conversion of one micromole of substrate per minute per gram under the specified conditions of the assay method.

2.6 Microwave treatment of the waste

Microwave pretreatment of kitchen waste was carried out for 30 min. The power of the microwave was set to 90 W. The study was performed on MS1927C LG microwave. After the treatment process, the powder is subjected to liquefaction/saccharification. Microwave-treated and untreated kitchen waste was subjected to Fourier transform infrared (FTIR) spectroscopy.

2.7 FTIR-ATR spectroscopic characterization

FTIR-ATR spectroscopic characterization was carried out for microwave-treated and untreated kitchen waste. Sample was ground to obtain fine powder. FTIR analysis was carried out with Bruker ATR Model alpha at Chandigarh College of Pharmacy, Landran, Mohali. Overhead ATR accessory was used with sampling station. The ATR diamond was carefully cleaned with pure isopropanol between crystal measurements. A 10-mg dry sample was carefully placed on the surface of the diamond crystal and each spectrum was absorbed to less than 100 N. Spectra were scanned at 4 cm^{-1} resolution from 4000 to 650 cm.

2.8 Modeling of liquefaction/saccharification process using response surface methodology (RSM)

Microwave-pretreated kitchen waste was proceeded to enzymatic saccharification with *Bacillus licheniformis* amylase. RSM was utilized for the modeling of experimentation. Effect of three parameters, viz. pH, concentration of dry kitchen waste and amylase concentration, on reducing sugar and ethanol production was studied. The model was constructed at α ± 1.68. A total of 20 runs were generated by using design expert software version 10. The implication of the model was carried out by performing analysis of variance (ANOVA) test. *p* value determines the significance of each coefficient unit. Multiple regression analysis was done by the software and is used to construct 2D contour plots. The model fitting was determined by regression coefficient R^2. A polynomial equation was constructed based on the response outcomes and was used to find out the optimum parameter setting for the determination of response. Multi-response optimization of bioethanol production and reducing sugar was carried out by numerical optimization software of Design Expert (Statease 9.0.7.1). The responses were set to maximum, and all the variables were kept in range. Optimal solutions were generated by the software according to the desirability in decreasing order. To validate the predicted set of conditions, pretreatment was done under optimized conditions and ethanol production and reducing sugar were measured.

2.9 Enzymatic liquefaction and saccharification of kitchen waste

Enzymatic liquefaction/saccharification of microwave-treated waste was done at an initial concentration of 40% w/v dry material for 8 h with 15 IU/g dry material amylase from *B. licheniformis* MTCC 1483 at 50 °C. 50 mM phosphate buffer was used to adjust the pH to 7.5. (Initial pH of the waste was 6.7.) Whole slurry was utilized for the fermentation experiments. Samples were also taken to quantify the reducing sugars.

2.10 Ethanol fermentation

Fermentation was performed in 500-ml Erlenmeyer flasks at 34 °C in solid-state conditions (SSF). Dry baker's yeast was added to whole slurry (consisting of 40% dry material saccharified with 15 IU/g amylase for 8 h, total reaction volume of 100 ml) at a concentration of 15 mg/g of initial dry material [26]. After 48 h, the broth was filtered, centrifuged and analyzed for ethanol concentration. Ethanol was estimated by standard potassium dichromate method [27]. The reducing sugar content of the fermented and unfermented kitchen waste was calculated by dinitrosalicylic acid method [25].

2.11 Process economics of bioethanol production

The cost involved in bioethanol production using kitchen waste was calculated by following the method of Osma et al. [28]. The cost involved is divided into three parts: cost of chemicals (C_{CM}), cost of equipments (C_{Eq}) and operating cost (C_{op}). For determining the C_{CM}, prices of reagents were taken from Sigma-Aldrich (St. Louis, USA) and HiMedia (India). The costs of equipment and operation (C_{Eq} and C_{Op}) were determined by taking a standard laboratory incubator (C_I), microwave (C_M) autoclave (C_A) and centrifuge (C_C). Their lifetime (LT) was taken as equal to their warranty period, one year for the incubator and centrifuge and two years for the autoclave and microwave. The energy consumption and efficiency of these devices were calculated according to the manufacturer's description. The equipment cost was calculated as per Eq. 1 as follows:

$$C_{\mathrm{Eq}} = C_{\mathrm{I}} + C_{\mathrm{A}} + C_{\mathrm{M}} + C_{\mathrm{C}} = \frac{D_{\max}/365}{\mathrm{Cap}_{\mathrm{I}}}\left(\frac{P_{\mathrm{I}}}{\mathrm{LT}_{\mathrm{I}}} + \frac{P_{\mathrm{A}}}{\mathrm{LT}_{\mathrm{A}}} + \frac{P_{\mathrm{M}}}{\mathrm{LT}_{\mathrm{M}}} + \frac{P_{\mathrm{C}} \cdot \mathrm{Cap}_{\mathrm{C}}}{\mathrm{LT}_{\mathrm{C}}}\right) \quad (1)$$

where $D_{\max}$ is the incubation period for obtaining maximum ethanol; $\mathrm{Cap}_{\mathrm{I}}$ is the capacity of the incubator; P_{I}, P_{A}, P_{M} and P_{C} are the prices of incubator, autoclave, microwave and centrifuge, respectively; LT_{I}, LT_{A}, LT_{M} and LT_{C} = lifetimes of incubator, autoclave, microwave and centrifuge, respectively.

As ethanol fermentation was carried out in solid state, a simple laboratory incubator was used for cost calculation. The operating cost was calculated by taking energy consumption (E) under consideration as follows:

Operating cost was calculated as follows:

$$C_I = \frac{E_I \cdot \left(24 \cdot D_{\max}\right)}{\mathrm{Cap}_I} \quad (2)$$

$$C_A = \frac{E_A}{\mathrm{Cap}_A} \quad (3)$$

$$C_M = \frac{E_M}{\mathrm{Cap}_M} \quad (4)$$

$$C_C = \frac{E_C}{\mathrm{Cap}_C} \quad (5)$$

where E_{I} energy consumption of incubator, E_{A} energy consumption of autoclave, E_{M} energy consumption of microwave and E_{C} energy consumption of centrifuge.

Bioethanol production cost ($\mathrm{Cost}_{\mathrm{Bet}}$) was calculated as in Eq. 6:

$$\mathrm{Cost}_{\mathrm{Bet}} = \frac{C_{\mathrm{CM}} + C_{\mathrm{Eq}} + C_{\mathrm{Op}}}{\mathrm{Beta}_{\max}}. \quad (6)$$

3 Statistical analysis

All the experiments performed in the present study were done in triplicates. Statistical analysis was carried out by using Sigma Stat version 12. Analysis of variance was applied with $p < 0.05$. The results shown in tables are given with standard deviation at 95% confidence interval.

4 Results and discussion

4.1 Characterization of kitchen waste

Feasibility of kitchen waste for bioethanol production is always a big challenge for biofuel industry [29]. The major problem is its non-standard nature compared to other standard lignocelluloses. Ethanol conversion potential of kitchen waste is highly dependent on its composition. Composition of kitchen waste varies according to season, food habits in a particular region and daily utilization of vegetables and fruits [30]. Therefore, the characterization of kitchen waste is must before its conversion to ethanol. In the present study, waste was collected for 30 days and then dried and ground into fine powder to minimize the variation in composition. The characteristics property of the kitchen waste is given in Table 1. The pH of waste was found to be 6.7. This pH was well suited for enzymatic treatment by amylase to breakdown the carbohydrate into simple sugars. Total organic carbon content of waste was high representing the presence of biodegradable carbohydrates, proteins and smaller lipids. This makes the kitchen waste a potential candidate to be utilized as raw material for bioethanol production. Volatile solids (VS) represent biodegradable components in the waste [31]. The VS value of 88.80% suggests that the kitchen waste contains high amount of biodegradable materials which can be utilized for bioethanol generation. C/N ratio of fermentation medium plays a vital role in bioethanol production. A low ratio will result in decreased yield, while a high ratio will result in high yield [32]. The C/N ratio of 33.47 obtained in the present study is good enough to yield maximum ethanol. A ratio greater than 35 results in accumulation of ammonia and volatile fatty acids which may interfere with bioethanol production by inhibiting the glycolytic pathway [31]. Volatile fatty acids also decrease the pH of the waste, resulting in low activity of enzymatic

Table 1 Characteristics of kitchen waste

Parameters	Weight fraction
pH	6.7
Total solids	14.04%
Total volatile solids	88.80%
Moisture content	88%
Ash content	11.08%
Total carbohydrates	62%
Total proteins	7.2%
Total organic carbon	50.2%
Kjeldahl N	1.5%
C/N ratio	33.47

hydrolysis. Therefore, the correct balance between C/N ratio is required for maximum bioethanol production.

4.2 Microwave pretreatment of waste

Kitchen waste was pretreated with microwave radiation for 30 min at a constant power of 90 W. Microwave heating is more energy efficient than conventional heating as the heat is penetrated equally through the entire volume of material [33]. However, high power of microwave creates hot spots of temperature in the waste material resulting in dehydration of carbohydrates. This leads to formation of furfurals and 5-hydroxymethylfurfural which are fermentation inhibitors [33]. Therefore, microwave treatment was carried out at low power. Low-power treatment resulted in breakdown of hydrogen bonds in carbohydrates as can be depicted in the infrared spectra of untreated waste and microwave treated waste in Fig. 1.

It can be observed from Fig. 1 that there was a sequential increase in the intensity of all the bands when microwave treatment was given to the waste sample. Microwave pretreatment resulted in the generation of bands at 3615 and 3499 cm^{-1} (Fig. 1). These bands are not observed in the untreated waste (Fig. 1). The band around 3610–3645 cm^{-1} is due to free –OH group. The results depicted that microwave treatment resulted in breakdown of sugar molecule leading to free –OH stretching. The band around 3401–3368 cm^{-1} is the absorption peaks of hydroxyl groups of the intramolecular hydrogen bond of cellulose. Microwave treatment resulted in the breakdown of cellulosic material, making it more accessible to enzymatic treatment. The band around 1016–1045 cm^{-1} shows the deformation of carbohydrates. From Fig. 1, it can be observed that the relative absorbance of carbohydrates increases with microwave treatment of kitchen waste.

Mikulski and Klosowski [33] carried out microwave-assisted dilute acid pretreatment of wheat and rye stillage at 300 W for 15 min. They obtained a final ethanol yield of 20 g/L after 48 h. Conesa et al. [34] had performed microwave pretreatment of pineapple industry waste and optimized the treatment conditions at different power for different time. They reported the microwave increased sugar yield up to 6.375 W/g power. Higher power and longer treatment resulted in sugar degradation. They also confirmed the presence of fermentation inhibitors at high microwave power. Pooja et al. [35] accomplished microwave-assisted acid/alkali pretreatment at 300 W for 7 min of agricultural residues of cassava. They suggested that microwave treatment is the most efficient pretreatment method. In another study, Zhang et al. [36] carried out microwave pretreatment at 80 W for 40 min assisted with 3% sulfuric acid of kitchen waste and concluded that the treatment resulted in 47.1% increase in sugar yield. Thus, microwave treatment at low power is an efficient method for sugar recovery from lignocellulosic waste.

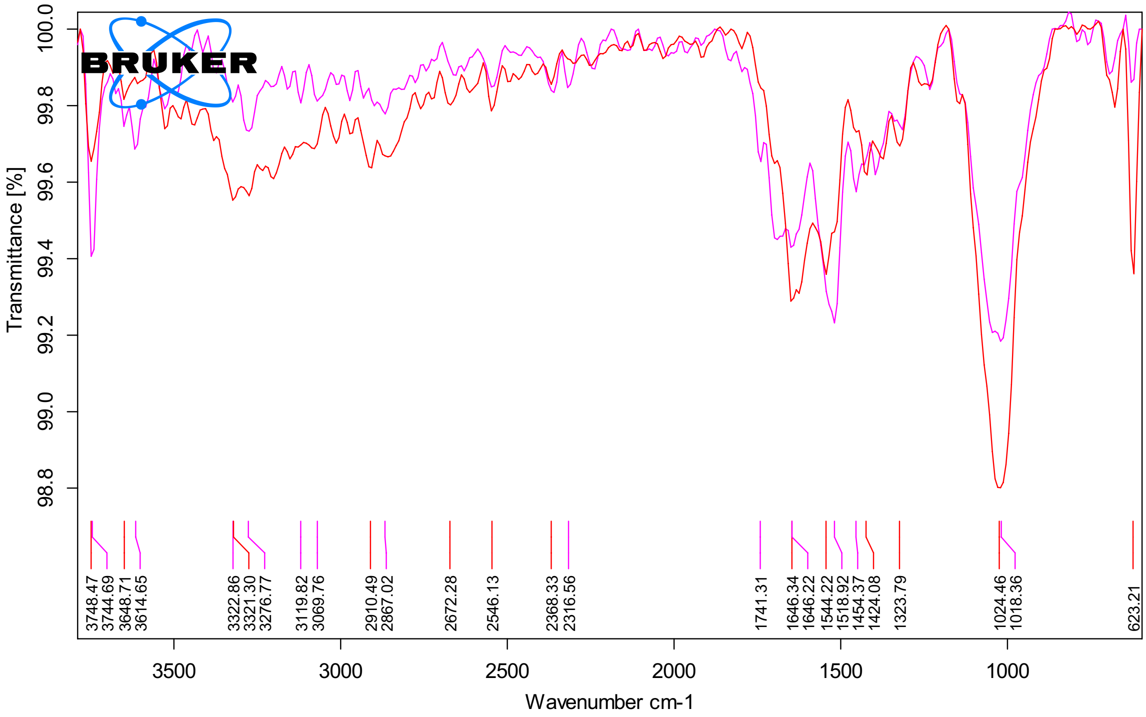

Fig. 1 FTIR-ATR profile of kitchen waste

There is no report in the literature where ethanol production was done without acid/alkali treatment.

4.3 Modeling of ethanol production by RSM

Kitchen waste is mainly composed of peels of potatoes, onions and other vegetables. These components are rich in starch. Amylase is an enzyme which breaks down starch by hydrolyzing α-(1–4) bond [37]. An enzymatic liquefaction/saccharification process is usually applied before fermentation to release soluble sugars. Moreover, at high dry material concentration, fermentation becomes difficult as the solution becomes more viscous [14]. Therefore, enzymatic saccharification of kitchen waste was performed with amylase to reduce the viscosity of the substrate and facilitate fermentation. Amylase treatment resulted in reduced viscosity as well as conversion of starchy sugars into glucose.

Under preliminary condition, 45% (w/v) dry material was used for ethanol fermentation to which 5 IU g^{-1} of amylase was added for enzymatic treatment. The treatment was carried out overnight. At initial setup, ethanol yield obtained was 49.72 g l^{-1}. To further increase the yield of ethanol, statistical optimization was done. Response surface methodology (RSM) was used to model the experiments. pH, substrate and enzyme concentration are the key factors which can affect the saccharification process and fermentation outcome [38, 39]. Although the optimum pH of incubation should be close to the optimum pH of activity of amylase, pH was chosen as one of the factor for the design of experiments. It was expected that the complex nature of kitchen waste can change the optimum pH of saccharification by this enzyme. Therefore, the pH was varied to study its effect on sugar release and ethanol recovery to study its interactive effect with other parameters under study. The optimum pH of *B. licheniformis* amylase activity was 8.0 [40]; however, optimum pH for the production of ethanol was observed to be 7.5 in the present study. Therefore, interactive effect of these variables on reducing sugar released and ethanol production was studied through RSM. The actual responses (reducing sugar and ethanol concentration) are presented in Table 2.

From Table 2, it was analyzed that maximum response of output parameters lies in central region. Increasing or decreasing the parameters under study results in decreased output responses. The results can be manifested to the fact that utilization of substrate at high dry mass concentration creates hindrance, resulting in improper digestion of substrate. Furthermore, ethanol yield and sugar release also reduce at high concentration of amylase which can be manifested to the fact that high concentration of enzyme can lead to enzyme dimerization which leads to a decrease in enzyme activity [41].

Table 2 Response surface design and outcomes for reducing sugar and ethanol production

Run	Space type	A: substrate concentration %	B: amylase IU/g	C: pH	Reducing sugar (microgram) µg/ml	Ethanol concentration(g/L)
1	Axial	48.409	15	7.5	240	65
2	Factorial	45	10	8	50	47
3	Factorial	35	20	8	355	59
4	Axial	40	23.409	7.5	294	22
5	Factorial	35	10	8	363	63
6	Factorial	35	20	7	43	10
7	Axial	40	15	8.3409	407	108
8	Axial	40	6.59104	7.5	253	23
9	Axial	40	15	6.6591	350	67
10	Factorial	35	10	7	395	76
11	Factorial	45	10	7	309	43
12	Center	40	15	7.5	460	127
13	Factorial	45	20	7	360	45
14	Axial	31.591	15	7.5	250	55
15	Factorial	45	20	8	445	106
16	Center	40	15	7.5	460	127
17	Center	40	15	7.5	462	133
18	Center	40	15	7.5	463	131
19	Center	40	15	7.5	465	133
20	Center	40	15	7.5	460	127

Many authors have utilized food waste or household waste for bioethanol production. Jung et al. [39] optimized the saccharification of food waste using RSM. They obtained 57.6 g/L ethanol at saccharification, pH of 5.20, enzyme reaction temperature of 46.3 °C, enzyme concentration of 0.16% (v/v), fermentation pH of 6.85, fermentation temperature of 35.3 °C and fermentation time of 14 h. Tang et al. [42] carried out the saccharification of kitchen waste by using Nagase N-40 glucoamylase and obtained 85.5% recovery of glucose. Use of flocculating yeast strain KF-7 in continuous fermentation led to 24 g/L of ethanol yield. Rahman et al. [26] obtained an ethanol yield of 7.3% v/v from kitchen waste without optimization. Matsakas et al. [14] also utilized household food waste at high dry material consistency and performed saccharification with amylase. They achieved an ethanol yield of 107.58 g/kg dry material. In a recent study, Prasoulas et al. [43] carried out saccharification of food waste using an enzymatic cocktail from *Fusarium oxysporum* F3. They obtained a final ethanol yield of 30.3 g/L. Hafid et al. [44] optimized the saccharification of kitchen waste by considering pH, temperature, glucoamylase activity, kitchen waste loading and hydrolysis time as significant parameters. They obtained sugar recovery of 62.71 g/L after 10 h.

4.4 ANOVA for response surface methodology for reducing sugar

Table 3 depicts the analysis of variance (ANOVA) for the response surface model of reducing sugar. It was found from the ANOVA that except substrate concentration, all other factors are found to be significant. Overall, the model is significant. The following model equation was obtained:

$$\begin{aligned}\text{Reducing Sugar }(\mu g) &= +461.64 - 0.65 * A + 11.35 * B \\ &\quad + 14.78 * C + 100.75 * \mathrm{AB} - 56.75 * \mathrm{AC} + 86.00 * \mathrm{BC} \\ &\quad - 76.42 * A2 - 66.34 * B2 - 29.22 * C2\end{aligned} \tag{7}$$

4.5 ANOVA for response surface methodology for ethanol production

ANOVA for the ethanol production is shown in Table 4. All terms are significant for ethanol yield. The following quadratic model equation was obtained for ethanol production:

$$\begin{aligned}\text{Ethanol Concentration }(\%) &= +66.27 + 1.86 * A - 0.49 * B + 6.32 * C \\ &\quad + 8.38 * \mathrm{AB} + 1.88 * \mathrm{AC} + 7.63 * \mathrm{BC} \\ &\quad - 12.23 *^2 - 18.95 * B^2 - 7.28 * C^2\end{aligned} \tag{8}$$

4.6 Interaction between variables

The two variables' interaction for reducing sugar and ethanol concentration is presented in Figs. 2 and 3, respectively, as contour graph. Figures 2a and 3a represent the effect of varying substrate and amylase concentration on reducing sugar and ethanol yield while keeping pH as constant. It was observed from Fig. 2a that an increase in substrate and amylase concentration increases the reducing sugar production until central value and after that a further increase in concentration resulted in less release of sugars. Similar results can be inferred from Fig. 3a as ethanol production is directly related to amount of reducing sugar released. It can be due to the fact that high dry

Table 3 ANOVA for response surface model

Source	Sum of squares	df	Mean square	*F* Value	*p* value prob > *F*	
Model	3.092E + 005	9	34,356.11	2680.31	< 0.0001	Significant
A: substrate concentration	5.69	1	5.69	0.44	0.5202	
B: amylase	1758.13	1	1758.13	137.16	< 0.0001	
C: pH	2983.73	1	2983.73	232.78	< 0.0001	
AB	81,204.50	1	81,204.50	6335.22	< 0.0001	
AC	25,764.50	1	25,764.50	2010.03	< 0.0001	
BC	59,168.00	1	59,168.00	4616.03	< 0.0001	
A^2	84,162.60	1	84,162.60	6565.99	< 0.0001	
B^2	63,431.53	1	63,431.53	4948.65	< 0.0001	
C^2	12,305.17	1	12,305.17	959.99	< 0.0001	
Residual	128.18	10	12.82			
Lack of fit	106.85	5	21.37	5.01	0.0508	Not significant
Pure error	21.33	5	4.27			
Cor total	3.093E + 005	19				

Table 4 Analysis of variance for response surface model for ethanol concentration

Source	Sum of squares	df	Mean square	*F* Value	*p* value prob > *F*	
Model	8678.14	9	964.24	346.10	< 0.0001	Significant
A: substrate concentration	47.27	1	47.27	16.97	0.0021	
B: amylase	3.27	1	3.27	1.17	0.03041	
C: pH	545.57	1	545.57	195.82	< 0.0001	
AB	561.13	1	561.13	201.41	< 0.0001	
AC	28.13	1	28.13	10.10	0.0099	
BC	465.13	1	465.13	166.95	< 0.0001	
A^2	2156.57	1	2156.57	774.07	< 0.0001	
$_B2$	5175.37	1	5175.37	1857.63	< 0.0001	
C^2	764.44	1	764.44	274.39	< 0.0001	
Residual	27.86	10	2.79			
Lack of fit	16.53	5	3.31	1.46	0.3445	Not significant
Pure error	11.33	5	2.27			
Cor total	8706.00	19				

mass concentration of substrate results in improper digestion of substrate leading to low recovery [14]. Figures 2b and 3b represent the combined effect of varying pH with substrate concentration keeping concentration of amylase as constant. Increasing pH from 6 to 7.5 increased the sugar yield, while at pH 8.0 there was a decrease in the response for reducing sugar (Fig. 2b), while the ethanol yield increases with an increase in pH from 7 to 8 (Fig. 3b). Figures 2c and 3c represent the effect of varying pH and amylase on reducing sugar and ethanol yield while keeping substrate concentration as constant. It can be observed that increasing pH and amylase concentration resulted in an increase in sugar and ethanol yield until cetral point and then decreased.

4.7 Validation of the predicted model

The response at the central point corresponds to a maximum degree of achievable ethanol concentration and reducing sugar for the three factors (Fig. 2). Therefore, increasing or decreasing the value of three parameters beyond the central limits will result in a decrease in ethanol concentration and reducing sugar. Thus, the central value from Table 2 was taken as optimum values, i.e., pH 7.5, dry material 40% (w/v) and amylase 15 IU/g. Experiments were performed under these sets of conditions. 129.67 gL^{-1}, i.e., 0.32 g ethanol per g biomass, was obtained at the end of the optimization process.

Several authors have made contribution in the field of bioethanol production. Gnansounou and Dauriat [45] produced 30.9 g bioethanol using 1 kg of kitchen waste. Moon et al. [8] obtained an ethanol yield of 29.1 gL^{-1} using food waste treated with carbohydrases and amyloglucosidases. Kim et al. [46] utilized starchy food waste treated with amylase yielding 57.5 gL^{-1} ethanol production after 14 h. Uncu and Cekmecelioglu [47] treated food waste with amylase and achieved 32.2 gL^{-1} ethanol production after 59 h of fermentation. Walker et al. [48] utilized starch-containing food saccharified with amylases yielding an overall ethanol content of 375 8 gL^{-1}. Jeong et al. [49] obtained an ethanol yield of 40.59 gL^{-1} after 24 h of fermentation. Cekmecelioglu and Uncu [50] obtained an ethanol yield of 23.3 gL^{-1} after 48 h of cultivation. In a recent study, Promon et al. [51] have treated vegetable peels with *B. subtilis* cellulase and optimized ethanol fermentation. It was observed that at 30 °C and pH 6.0 maximum ethanol percentage obtained after 48 h was 14.17%. In a recent study, ethanol yield of 30.8 gL^{-1} was achieved by using food waste as substrate using in-house developed enzymes for hydrolysis [43]. By comparing the results from the literature, it was concluded that ethanol production efficiency (129.27 g/L) obtained during this work was higher than that compared to other work reported in the literature. The advantage of using kitchen waste with microwave technology also reduces the cost of overall treatment process. This fact was validated by the process economic study done in the next section.

4.8 Process economics for bioethanol production

Bioethanol is a sustainable alternative to gasoline and is based on green technology. But the cost of production always remains a problem for the successful implementation of a process at industrial scale. The cost of producing first generation ethanol is expectedly high because of the high price of raw material [52]. Second-generation ethanol utilizes waste of no value resulting in steep decrease in prices [52]. However, the cost of

Fig. 2 Two-dimensional contour graph showing the relationships between variables and reducing sugar

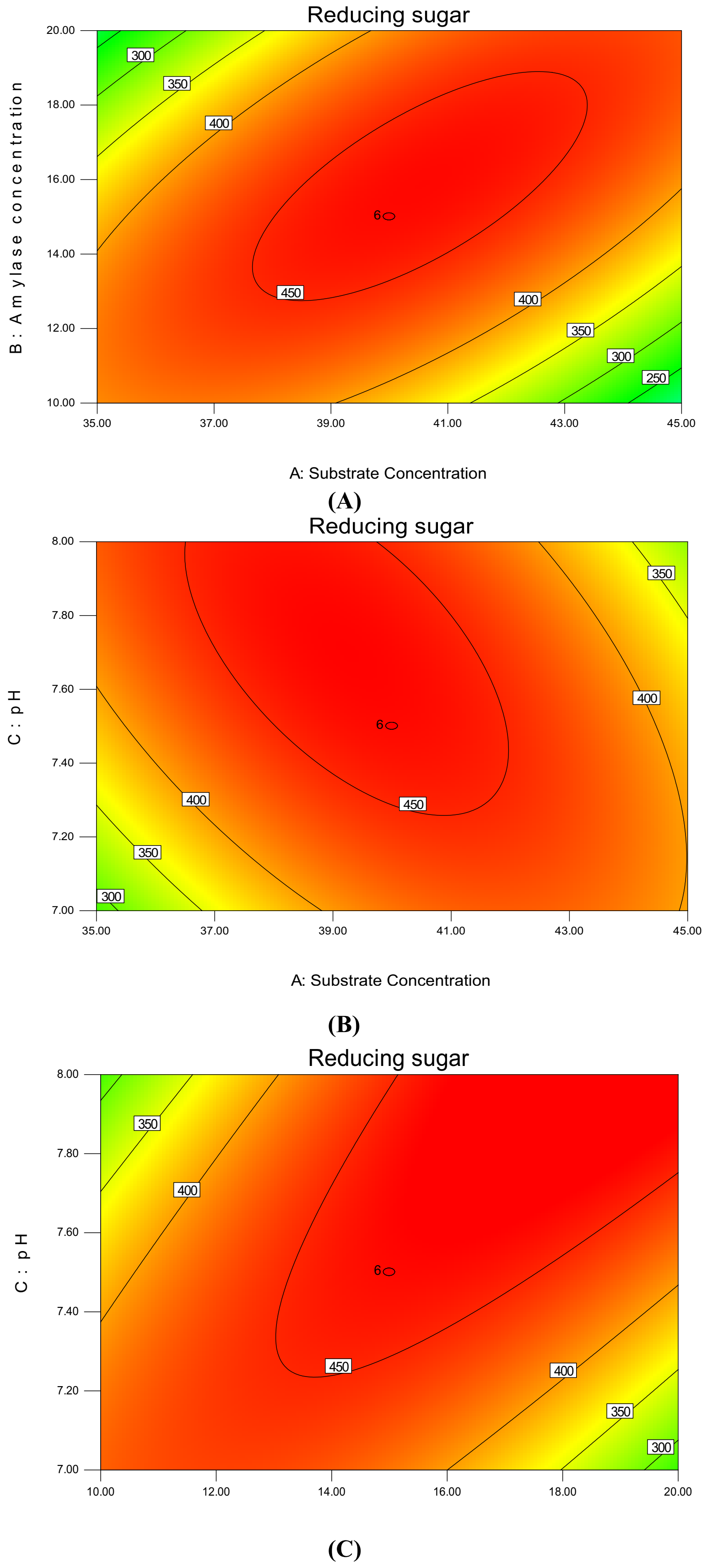

Fig. 3 Two-dimensional contour graph showing the relationships between variables and ethanol concentration

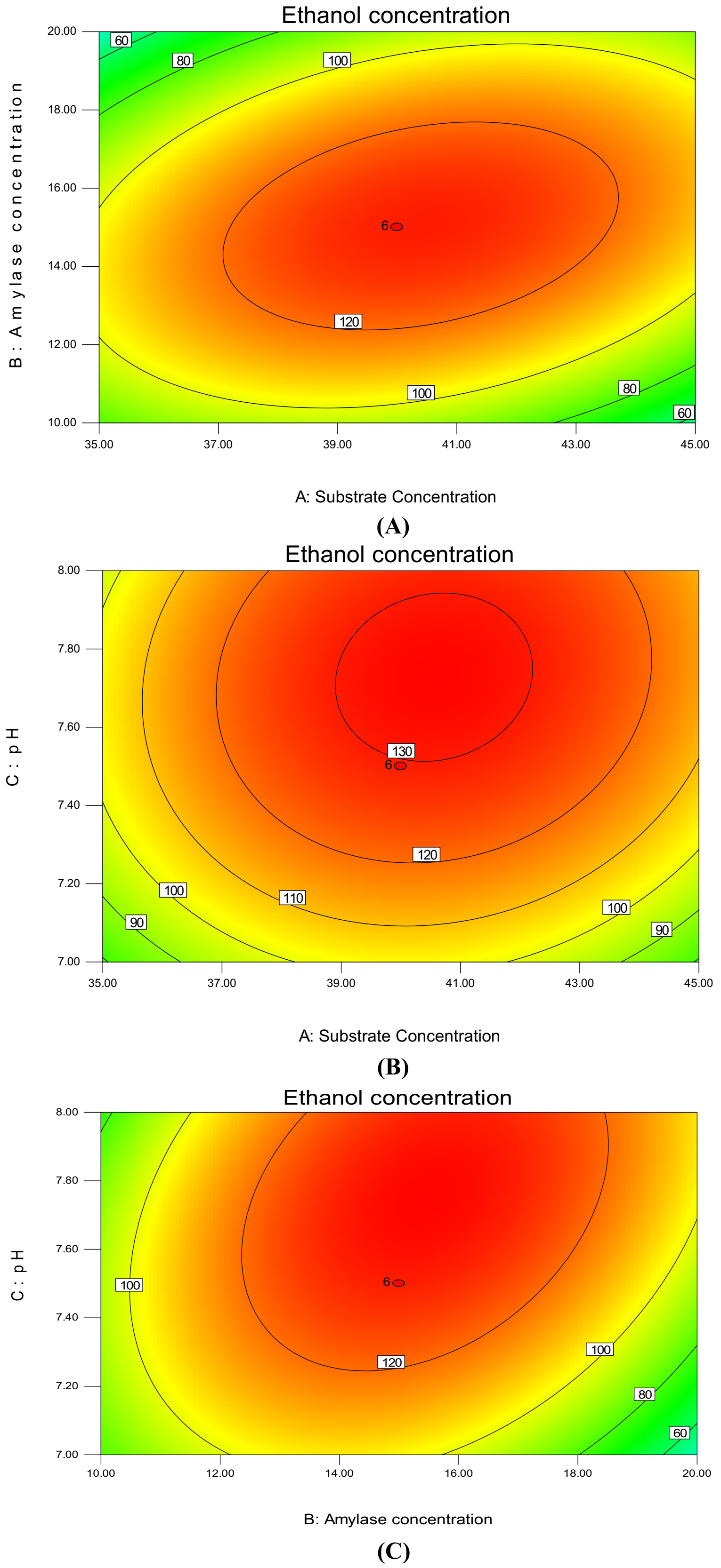

Table 5 Cost analysis of bioethanol production

Factors	Cost
Total bioethanol yield (IU) (Bet_{tot})	0.129 g/ml
Cost of chemicals [C_{CM} (Rs)]	0.072/-
Cost of equipment [C_{Eq} (Rs)]	0.00644/-
Operating cost [C_{Op} (Rs)]	0.00183/-
Total cost (Rs)	0.08027/-
Final price (Rs)	0.62 Rs per g bioethanol
MSEP*	0.143$/l ethanol

*MSEP, minimum selling ethanol price

pretreatment (acid/alkali) and the market value of commercial enzyme make the process of conversion of lignocellulosic waste into ethanol economically unfeasible. In this study, we reported a simple physical method of biomass pretreatment by microwave without acid and alkali treatment. Furthermore, the in-house developed enzyme was utilized in the present study. Process economics for the bioethanol production was calculated by considering the cost of chemicals, cost of equipments and operating cost. The cost involved was calculated as cost per unit volume of bioethanol produced (Table 5). As kitchen waste was used as raw material for fermentation, the cost of raw material is negligible. The cost of equipment included all instruments used for upstream and downstream processing. Among the total cost, the cost of chemicals accounts for 89% of total cost which mainly involves cost of chemicals used for amylase production. The cost of instruments accounts for 8% of the total cost. However, the cost of equipment is one time cost and subsequently only maintenance cost is required. Total operating cost is just 3% of the total cost.

The cost of ethanol production came out to be 0. 062 (Rs/g l^{-1}) in the present study. The minimum ethanol selling price (MESP) of gasoline is 4.52 $/gal ethanol, i.e., 1.19 $/l [53]. Corn ethanol's, the first-generation ethanol, selling price is around 8 $/l of ethanol [52]. Prices of corn are increasing day by day, thus increasing the MESP for corn ethanol. Muhammad and Rosentrater [54] reported MESP of 0.64 $/L of ethanol produced from fermentation of food waste. 127.29 g/L, i.e., 163 ml/L of ethanol, was produced in the present study. The cost of bioethanol produced in the present study was calculated as 0.143 $/l ethanol. Thus, a 8.32-fold decrease in production cost from the cost of gasoline was achieved. Thus, the present study revealed the potential of utilizing kitchen waste for ethanol production at industrial scale.

5 Conclusion and future prospects

Bioethanol is an alternative to fossil fuel and can be used as biofuel. In the present study, bioethanol was produced from kitchen waste. Microwave was used to treat the waste followed by enzymatic liquefaction and saccharification by amylase. No alkali/acidic treatment was carried out. The final yield of 129.27 gl-1of ethanol costing around 0.00088$ obtainable in this study makes the entire process economically feasible and industrially viable.

The waste or the stillage generated as fermentation by-product has many potential uses which was not explored in the present study. The whole stillage, i.e., the liquid by-product of fermentation industry, contains fibers, proteins, fats and dead yeast cells. In the future, this stillage can be utilized for the generation of methane by anaerobic digestion. Methane produced can then be used as fuel. Alternatively, the whole stillage can be separated by centrifugation into liquid fraction and solid fraction. The solid fraction is rich in lignocelluloses and can further be used for the production of cellulosic ethanol.

6 Author contributions

SS has written and analyzed the manuscript. PSK has performed the experiments. MK helped in revising the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Availability of data and material Not applicable.

Ethical approval No animal work has been done in the manuscript.

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